AD-A265 732



| 1. AGENCY USE ONLY (Leave blank) | 2. REPORT DATE | 3. REPORT TYPE AND DATES COVERED |
|---|---|---|
| | 18 May 93 | ANNUAL/1 Mar 91 - 28 Feb 93 |

**4. TITLE AND SUBTITLE**
PHASE-SHIFTING EFFECT OF LIGHT AND EXERCISE ON THE HUMAN CIRCADIAN CLOCK

**5. FUNDING NUMBERS**
G AFOSR-90-0222
PE 61102.
PR 2312
TA CS

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**8. PERFORMING ORGANIZATION REPORT NUMBER**
AFOSR-TR- 93 0 3

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**10. SPONSORING / MONITORING AGENCY REPORT NUMBER**



**11. SUPPLEMENTARY NOTES**

**12a. DISTRIBUTION / AVAILABILITY STATEMENT**
Approved for public release;
distribution unlimited

**12b. DISTRIBUTION CODE**



**13. ABSTRACT** (Maximum 200 words) 93 6 11 011

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| 14. SUBJECT TERMS | | | 15. NUMBER OF PAGES 17 |
|---|---|---|---|
| | | | 16. PRICE CODE |
| 17. SECURITY CLASSIFICATION OF REPORT (U) | 18. SECURITY CLASSIFICATION OF THIS PAGE (U) | 19. SECURITY CLASSIFICATION OF ABSTRACT (U) | 20. LIMITATION OF ABSTRACT UNLIMITED |

Revised April 5, 1993.

# PRELIMINARY STUDIES ON THE PHASE-SHIFTING EFFECTS OF LIGHT AND EXERCISE ON THE HUMAN CIRCADIAN CLOCK

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Running title: Phase-shifting human rhythms

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10 pages
3 figures
one table

ABSTRACT

The aim of the present study was to determine the magnitude and direction of immediate phase-shifts of human rhythms following a single exposure to a 3-hour pulse of bright light or physical activity. The pulse of light or activity was presented under "constant routine" conditions and measurement of the resultant phase-shifts were performed under the same constant routine conditions on the first day following pulse presentation. Four overt rhythms which are strongly dependent on circadian timing, i.e. the rhythms of plasma cortisol, plasma TSH, plasma melatonin and body temperature, were monitored. Based on the analysis of the TSH profiles, our findings indicate that exposure to light around the time of the minimum of body temperature results in phase-advances averaging less than one hour in magnitude. Exposure to light approximately 3 hours before the time of the minimum of body temperature results in 1-2 hour phase-delays. Preliminary analyses of the melatonin profiles confirm these observations. Our findings regarding the effects of exercise are still inconclusive.

INTRODUCTION

Until approximately ten years ago, it was thought that, unlike all other mammalian species, the human circadian system was largerly insensitive to the light-dark cycle and that social cues were the major synchronizing agents. During the past decade, a number of detailed studies have demonstrated that light has major synchronizing effects on human rhythms. However, information regarding the basic mechanisms of photic control of the human circadian clock remains scarce. In particular, because the majority of studies examining the effects of exposure to light have involved repeated application of the stimulus (Broadway, et al., 1987; Czeisler, et al., 1986; Czeisler, et al., 1989; Daan and Lewy, 1984; Dijk, et al., 1989; Drennan, et al., 1989; Lewy, et al., 1987), it is still unclear whether human rhythms phase-shift in response to a single light pulse. Theoretical extrapolations from phase-shifts observed after exposure to a complex stimulus involving light, dark and sleep, have suggested that the human circadian system responds to light in a fashion different from that known in all other mammalian species, i.e. a single exposure to light would not be associated with consistent phase-shifts but would instead decrease rhythm amplitude(Czeisler, et al., 1989, Jewett et al, 1991). In contrast, a recent study examining the effects of a single exposure to light in the presence of sleep displacement reported phase-shifts of one to two hours, i.e. similar to those observed in animal species (Minors, et al., 1991). Similarly, phase advances of 1.2 to 2.6 hours of the nocturnal melatonin rise were observed on the first day after exposure to a single pulse of bright light given in the early morning hours (Buresova et al, 1991).

The evolution of concepts regarding zeitgebers for non-human mammalian rhythms has run in some ways opposite to that occurring in the field of human rhythms. Indeed, social and/or behavioral cues were long thought to be ineffective as zeitgebers in rodents and other mammals, but evidence has accumulated over the past few years to indicate that behavioral changes are indeed capable of inducing shifts in circadian rhythms. Specifically, stimuli which cause an alteration of the rest-activity cycle, either by eliciting activity during the normal rest period or by preventing activity during the normal active period, result in phase shifts of circadian rhythms (Turek, 1989; Mrosovsky et al., 1989). So far, there has been no systematic evaluation of the potential phase-shifting effects of single exposures to nighttime activity or daytime sleep in human volunteers.

The aim of the present study was to determine the magnitude and direction of immediate phase-shifts of human rhythms following a single exposure to a 3-hour pulse of bright light or physical activity. Our goals were to answer the two following major

questions regarding the control of the human circadian system: 1. does the human circadian clock phase-shift in response to a single exposure to light or, alternatively, does single exposure result in amplitude reduction without consistent phase-shifting ? 2. can physical activity have zeitgeber effects for human rhythms ? The pulse of light or activity was presented under "constant routine" conditions and measurement of the resultant phase-shifts were performed under the same constant routine conditions on the first day following pulse presentation. To improve the reliability and robustness of estimations of circadian phase positions and to increase the ability to discriminate peripheral effects on overt rhythms from central effects on the circadian clock, we monitored four overt rhythms which are strongly dependent on circadian timing, i.e. the rhythms of plasma cortisol, plasma TSH, plasma melatonin and body temperature. In addition, measures of sleepiness and cognitive performance were obtained at hourly intervals.

## SUBJECTS AND METHODS

### Subjects

Seventeen normal young men, ages 20-30 years, have been studied so far. All were non-obese and in good physical condition obtained through regular moderate exercise. None had a personal history of psychiatric illness, endocrine illness or sleep disorder. Positive criteria for selection included regular life habits and an habitual total sleep time of at least 7 hours. Shift workers and subjects having experienced a transmeridian flight less than 6 weeks prior to the start of the study were excluded. The protocol was approved by the Institutional Review Board and written informed consent was obtained from all subjects.

### Experimental protocol

Each subject participated in three separate studies, one baseline study with measurements of circadian phase positions in the absence of zeitgeber stimulus, one study with exposure to a 3-hour pulse of light and one study with exposure to a 3-hour pulse of exercise. The studies were separated by two weeks.

Each study was preceded by a 7-day period of entrainment to a fixed light-dark and sleep-wake cycle, including two days of habituation to the laboratory conditions. During this pre-study period, the volunteers were asked to comply with a standardized schedule of sleep in total darkness (23:00 to 07:00) and meal times and their activity-rest cycle was monitored via a wrist monitor. During the 2-day habituation period, the volunteers continued to wear the wrist activity monitors, and body temperature was recorded via a telemetry system (Cortemp, Human Technologies Inc., St Petersburg,

Florida). The 7-point Stanford Sleepiness Scale, the Digit-Symbol Substitution Test (1-min paper and pencil version) and the Symbol Copying Test (1-min paper and pencil version) were administered at hourly intervals.

After awakening from the second habituation night, the subjects had breakfast and this was their last meal until the end of the study. Measurements of sleepiness, tests of cognitive performance and monitoring of activity and temperature continued as during the habituation days. At 12:00 noon, a glucose infusion at a constant rate of 5g/kg/24 hours was started and the subjects were maintained on a regimen of bedrest with enforced wakefulness in dim indoor light (<300 lux). These conditions of constant light exposure, constant posture, constant wakefulness and constant caloric intake were maintained for 38 hours, i.e. until 02:00 two nights later, when the subjects were allowed to obtain recovery sleep. Constant glucose infusion continued until the end of recovery sleep. At 15:00, a sampling catheter was inserted in the other arm. Blood sampling at 20-min intervals started at 18:00 and continued without interruption for 37-39 hours, i.e. until the end of recovery sleep.

The subjects had access to external time cues (wristwatch, radio and television programs, social contacts) throughout the study. The constant routine conditions were enforced at all times during the baseline study. During the two other studies, the only deviations from constant routine conditions were during the 3 hours of exposure to bright light or exercise. During exposure to bright light, the subjects remained recumbent. During the exercise session, the subjects remained in dim light.

A schematic representation of the protocol as well as mean temperature and activity profiles obtained during the baseline studies are shown in Figure 1.

Stimulus exposure

An estimation of the subject's individual circadian phase was obtained during the constant routine performed under baseline conditions. As in previous human studies, the timing of the center of the stimulus was related to the estimated timing of the circadian temperature minimum. In 11 of the 17 subjects, the stimulus was timed to occur 0-2 hours after the estimated timing of the circadian temperature minimum. We hypothesized that, in the case of light, this timing of exposure would result in phase-advances and, accordingly, referred to these studies as the "phase advance protocol". In the other 6 volunteers, the stimulus was administered approximately 3 hours before the estimated timing of the circadian temperature minimum. We hypothesized that, in the case of light, this timing of exposure would result in phase-delays and, accordingly, referred to these studies as the "phase delay protocol". Light exposure was obtained by placing a mobile panel of fluorescent tubes providing a light intensity of 5,000 lux at two feet in front of the

subject. As in previous studies (Czeisler, et al., 1989, Jewett et al., 1991), each 3-hr period of exposure to 5,000 lux was bracketed by 30 min of 2,500 lux illumination. For exercise, a "high" and "low" workload on a stationary arm-and-leg exerciser were defined for each subject prior to the study in the cardiac laboratory. The workload and type of exercise were varied in 5 cycles of 36 minutes each, alternating cycles with high and low workloads (starting with high workload), and including 15 min of arm exercise, 15 min of leg exercise and 6 min of rest.

Hormonal assays

Plasma concentrations of cortisol and TSH were measured on each sample by radioimmunoassay. Melatonin determinations are currently in progress.

Estimation of circadian waveshapes and phase reference points

The waveshape of each individual profile of temperature and hormonal levels was quantitatively characterized by a best-fit curve obtained using a robust, locally weighed, regression procedure (Cleveland, 1979). The acrophases and nadirs were defined as, respectively, the times of occurrence of maxima and minima in the best-fit curve. The amplitude of the best-fit curve was defined as 50% of the difference between its maximum and its minimum. The nadir of the best-fit curve was used as the phase marker for the temperature rhythm. Definitions of phase markers for hormonal profiles took into account the specific secretory characteristics of each of the three hormones measured. For cortisol, which has a highly pulsatile secretory pattern, the phase marker was the onset of the rise towards the morning maximum, defined as the start of the first significant pulse after the nocturnal nadir. For TSH, which exhibits less secretory pulsatility, the phase marker was the onset of the rise towards the nocturnal acrophase, defined as the time when the best-fit curve reached the level corresponding to the nadir plus 50% of the amplitude. For melatonin, the phase marker to be used will be the onset of the nocturnal rise but precise mathematical definition awaits availability of complete laboratory results.

## RESULTS

The design of the present study involved two steps: 1. baseline estimations of the circadian phase position; 2. timed application of the stimulus relative to this baseline estimation in two separate studies for light and exercise, respectively. Four overt rhythms were used to obtain phase and amplitude estimations. Because these rhythms were monitored continuously for 38 hours, several markers of phase position could be observed under constant routine conditions before as well as after application of the stimulus in the same study. These markers include the onset of nocturnal TSH secretion (which normally occurs between 21:00 and 22:00), the onset of nocturnal melatonin

secretion (which normally occurs between 21:00 and 22:00) and the onset of cortisol secretion (which normally occurs around midnight). This design theoretically permits a within-subject and within-study comparison of phase and amplitude estimations derived from hormonal profiles before and after light exposure, exercise exposure or no stimulus (i.e. baseline conditions).

The temperature data served only to obtain a single estimation of circadian phase position in the baseline study because constant routine conditions could not be extended long enough to permit the observation of a second unmasked nocturnal nadir. Exercise, as expected, resulted in a marked temperature rise which precluded accurate phase and amplitude estimations from the temperature profile. Likewise, in about half of the recordings obtained in the studies with light exposure, the stimulus appeared associated with a temperature rise which corrupted phase and amplitude estimations. Thermal effects of light exposure have been previously observed by other investigators (Dijk et al., 1991).

Detailed examination of the cortisol data revealed that estimations of phase-shifts of the onset of the circadian rise following stimulus application could not be reliably interpreted. Indeed, in approximately half of the studies, the post-stimulus phase marker did not occur before the onset of recovery sleep. Furthermore, in the phase-delay protocol, the beginning of exposure to the stimulus preceded or roughly coincided with the "pre-stimulus" phase marker. Finally, contrary to current notions, exercise appeared to have some direct, i.e. masking, effects on cortisol secretion. Amplitude estimations derived from the cortisol profiles did not reveal amplitude changes following either light or exercise exposure.

The current status of data analysis thus rests on the examination of the TSH profiles. Figures 2 and 3 illustrate the mean TSH profiles obtained in the "phase-advance" and "phase-delay" protocols, respectively. The data are referenced with respect to the circadian temperature minimum rather than clock time. Table 1 summarizes the analysis of the TSH profiles. In response to light, significant phase-advances were observed when light was administered 0-2 hours after the temperature minimum and significant phase-delays were observed when light was administered 3 hours before the temperature minimum. Preliminary analyses of currently available melatonin profiles confirm these characteristics of this phase-response curve to light.

Unexpectedly, exercise was associated with a rapid and marked increase in TSH levels irrespective of the time of administration (Table 2). The nocturnal TSH rise on the second day was less marked than during the first part of the constant routine on all three experimental conditions. The simultaneous measurement of triiodothyronine (T3) on a

limited number of individual profiles indicated the existence of a nocturnal rise of T3 paralleling that of TSH during the first night of sleep deprivation. The reduction of the TSH rise on the second day was probably the result of the negative feedback by the elevated T3 levels on the first day. This dampening of the circadian TSH variation (which was not associated with a dampening of the cortisol rhythm) was more marked following exposure to exercise and therefore possible phase-shifts associated with exercise exposure could not be reliably evaluated. Limited data on the melatonin profiles suggest that exercise exposure induced significant phase-delays, irrespective of the timing of the pulse relative to the temperature minimum.

## DISCUSSION

The present preliminary results support the concept that photic control of the human circadian clock is qualitatively similar to that of other mammalian species, as previously suggested by Minors et al (1991) and Buresova et al (1991). Contrasting findings (Czeisler, et al., 1989; Jewett, et al., 1991) may be related to the presentation of multiple stimuli (i.e. light, dark and displaced sleep) within a given 24-hour cycle rather than to the single stimulus-single exposure paradigm used in the present study. Another possible explanation for the discrepancy may be related to the intensity of the light stimulus. According to Jewett et al (1991), observation of circadian amplitude reduction without consistent phase-shifts following light exposure is dependent on a "critically" timed stimulus of "critical" intensity. While the timing of the stimulus in the present study was similar to those explored in these previous reports, the intensity of light exposure may have been "sub-critical".

Findings regarding the possible phase-shifting effects of exercise remain inconclusive due to the unexpected direct effects of exercise on TSH secretion revealed in this study. Preliminary analyses of a limited number of melatonin profiles have shown the occurrence of phase-delays only.

ACKNOWLEDGEMENTS

We thank the volunteers who participated in this demanding protocol and the nursing staff of the General Clinical Research Center of the University of Chicago for their help and care. Mrs. William Pugh and P. Rue provided expert technical assistance with hormonal assays. These studies were supported by grant 90-022 from the US Air Force Office of Scientific Research and by grant USPHS MO1 RR00055 of the General Clinical Research Center of the University of Chicago.

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## Table 1 : TIMING OF ONSET OF TSH RISE

### 'PHASE ADVANCE' PROTOCOL

| | Day 1 | Day 2 | Paired t-test |
|---|---|---|---|
| **Baseline** | 21:27 ± 53 m | 21:22 ± 67 m | 0.82 |
| **Light** | 21:20 ± 61 m | 20:42 ± 79 m | 0.048 |
| **Exercise** | 21:47 ± 76 m | -* | na |

### 'PHASE DELAY' PROTOCOL

| | Day 1 | Day 2 | Paired t-test |
|---|---|---|---|
| **Baseline** | 21:13 ± 33 m | 20:57 ± 82 m | 0.579 |
| **Light** | 21:06 ± 71 m | 22:03 ± 73 m | 0.042 |
| **Exercise** | 21:40 ± 31 m | -* | na |

* The onset of the TSH rise could not be estimated on day 2 due to a marked dampening of the profile following exposure to exercise.
na: not available

Table 2 : POST-PRE STIMULUS RATIO OF MEAN TSH LEVEL

"PHASE ADVANCE" PROTOCOL

| | | |
|---|---|---|
| **Baseline** | 0.88 ± 0.12 | |
| **Light** | 0.87 ± 0.16 | |
| **Exercise** | 1.17 ± 0.19 | $p < 0.01$ as compared to B and L |

"PHASE DELAY" PROTOCOL

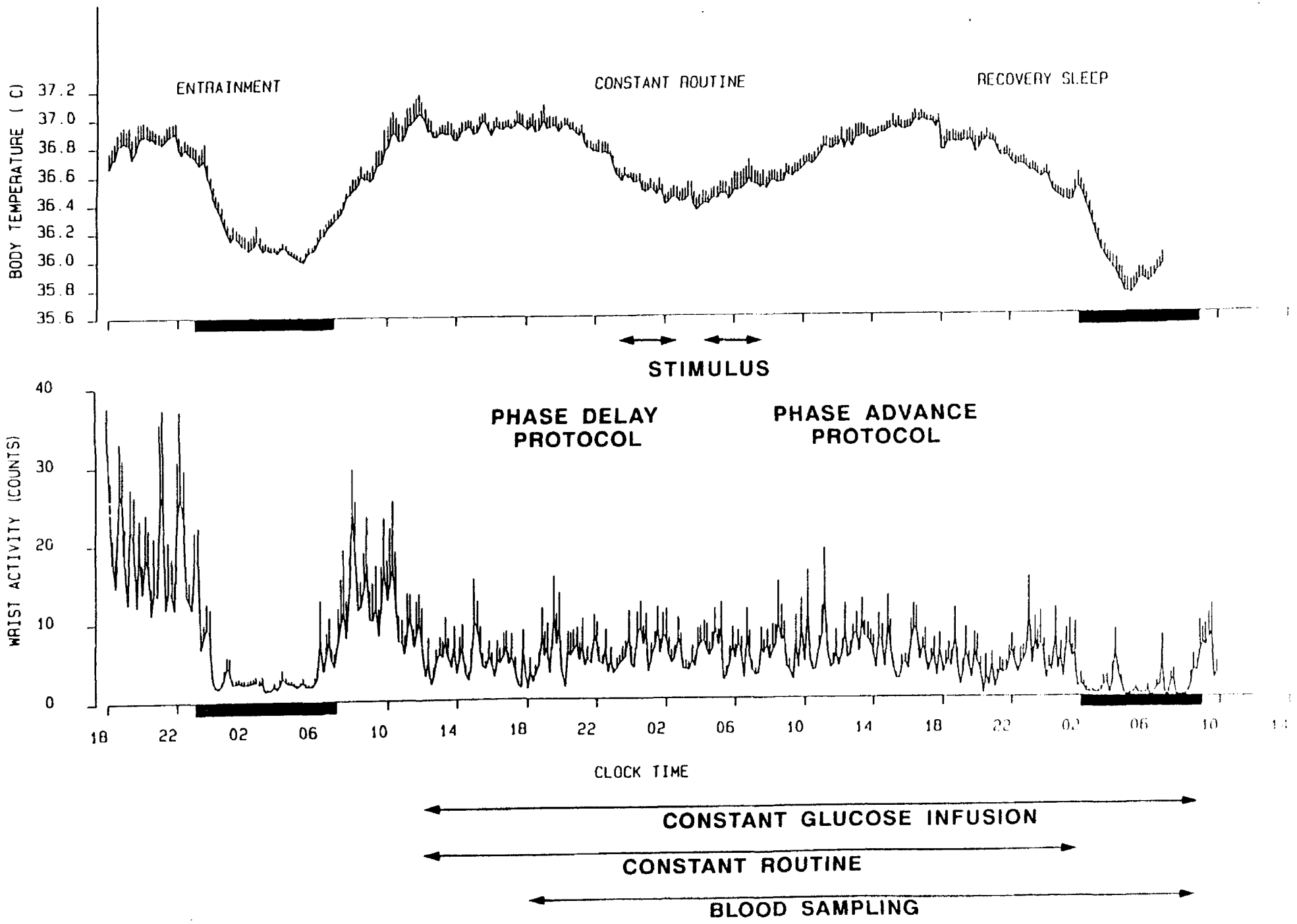
| | | |
|---|---|---|
| **Baseline** | 1.25 ± 0.17 | |
| **Light** | 1.41 ± 0.21 | |
| **Exercise** | 1.78 ± 0.37 | $p < 0.05$ as compared to B and L |

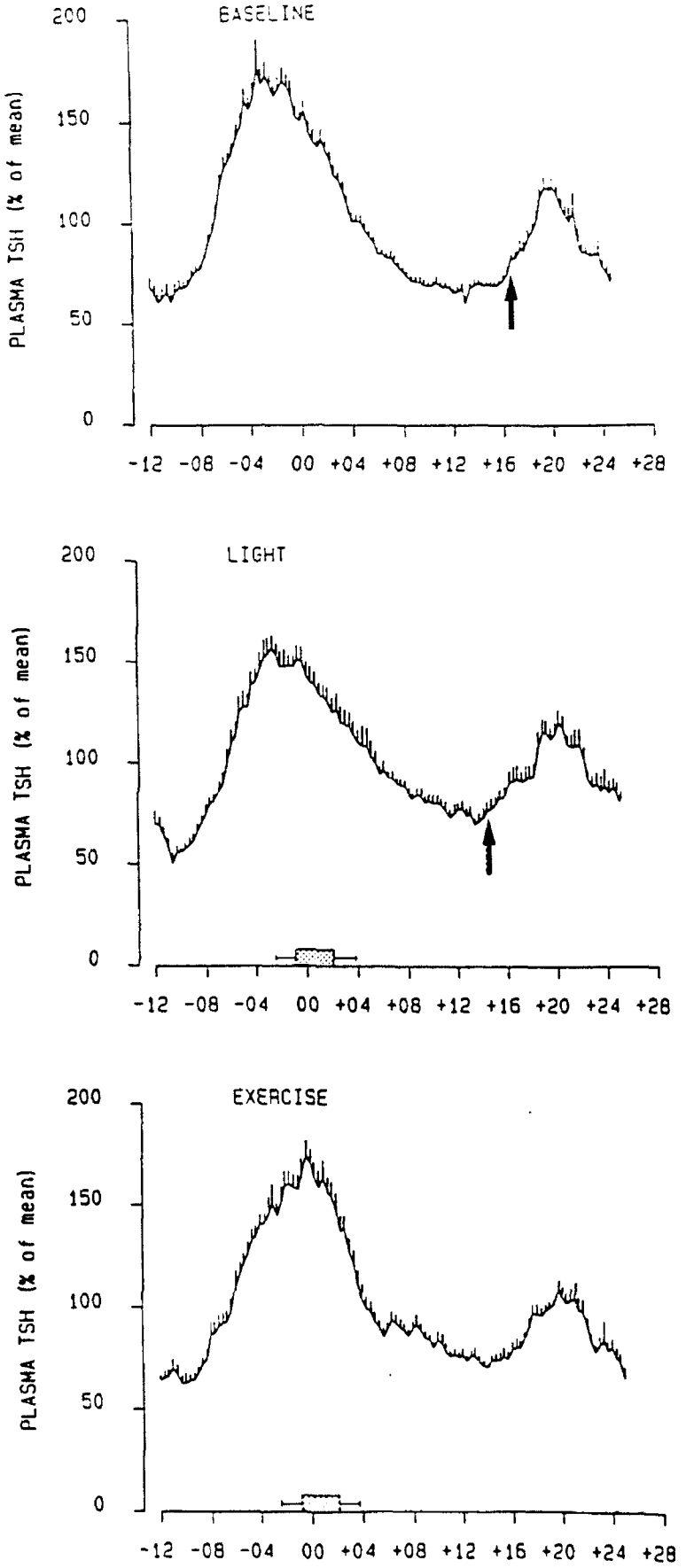
FIGURE LEGENDS

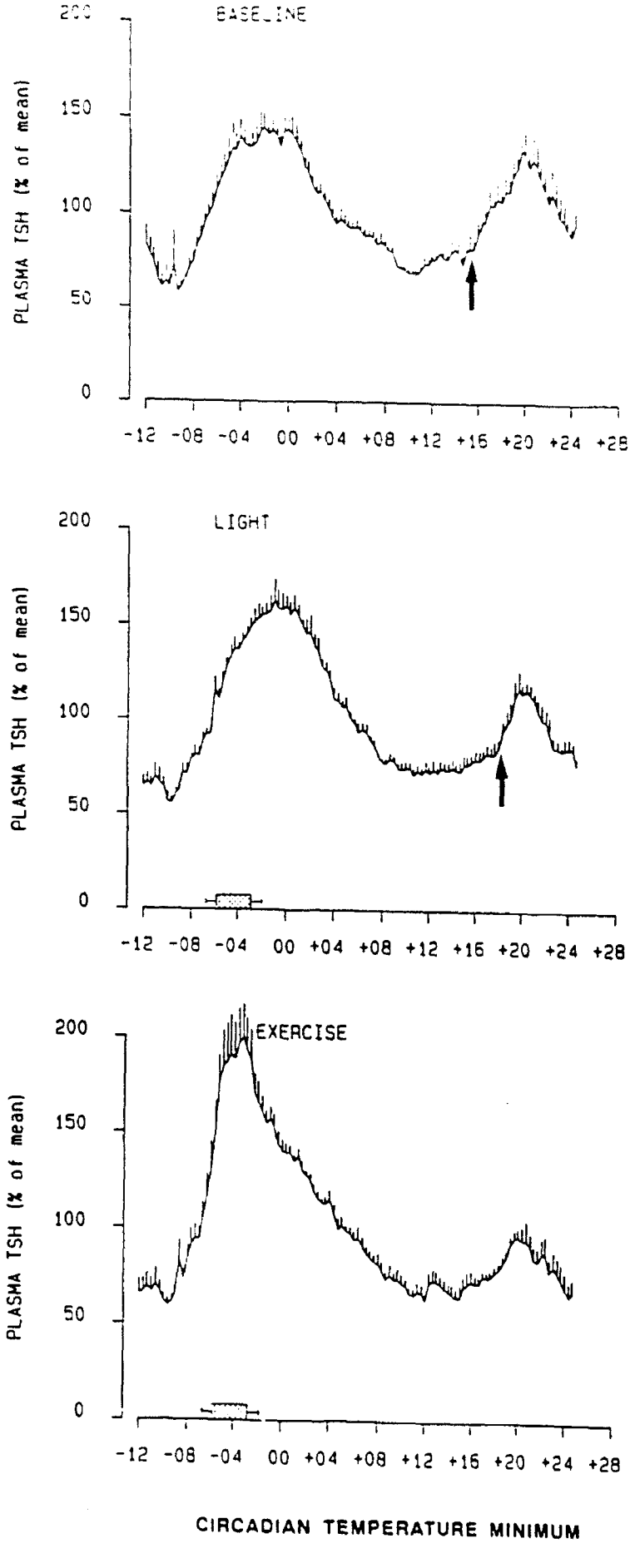
Figure 1: mean (+ SEM) recordings of body temperature (top) and wrist activity (bottom) observed in the first 8 volunteers during the baseline study. Black bars indicate the sleep periods. The periods of glucose infusion, blood sampling and enforcement of constant routine conditions are indicated by arrows. The range of timings of the center of the stimulus for the "phase advance" and "phase delay" protocol are also shown by arrows.

Figure 2: mean (+SEM) profiles of plasma TSH in the baseline study, the study with light exposure and the study with exercise exposure in the "phase advance protocol" (11 subjects). The average timing of stimulus presentation (± SD) is shown as a shaded area. The arrow indicates the mean timing of the circadian TSH rise.

Figure 3: mean (+SEM) profiles of plasma TSH in the baseline study, the study with light exposure and the study with exercise exposure in the "phase delay protocol" (6 subjects). The average timing of stimulus presentation (± SD) is shown as a shaded area. The arrow indicates the mean timing of the circadian TSH rise.


ENTRAINMENT
CONSTANT ROUTINE
RECOVERY SLEEP
BODY TEMPERATURE ( C)
37.2
37.0
36.8
36.6
36.4
36.2
36.0
35.8
35.6
STIMULUS
PHASE DELAY PROTOCOL
PHASE ADVANCE PROTOCOL
WRIST ACTIVITY (COUNTS)
40
30
20
10
0
18
22
02
06
10
14
18
22
02
06
10
14
18
22
02
06
10
CLOCK TIME
CONSTANT GLUCOSE INFUSION
CONSTANT ROUTINE
BLOOD SAMPLING


BASELINE
LIGHT
EXERCISE
PLASMA TSH (% of mean)
200
150
100
50
0
-12 -08 -04 00 +04 +08 +12 +16 +20 +24 +28
CIRCADIAN TEMPERATURE MINIMUM


BASELINE
LIGHT
EXERCISE
PLASMA TSH (% of mean)
200
150
100
50
0
-12 -08 -04 00 +04 +08 +12 +16 +20 +24 +28
CIRCADIAN TEMPERATURE MINIMUM